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Antennal transcriptome analysis and expression profiling of chemosensory protein genes in *Oedaleus asiaticus* (Orthopera：Acrididae)

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Abstract：【Aim】Chemosensory proteins (CSPs) are soluble carrier proteins that may play multiple roles in insects. The objective of this study is to assembly the antennal transcriptomes of *Oedaleus asiaticus*, one of the most important grasshopper pests in northern China, to identify the chemosensory protein genes, and to analyze their tissue expression profiles. 【Methods】The antennal transcriptomes of the *O. asiaticus* adults were sequenced and assembled by RNA-Seq. The CSP genes were identified by screening the transcriptome data, and their expression levels in different adult tissues (antenna, head without antennae and mouthparts, labrum, labium without labial palps, labial palp, maxillary palp, thorax, tarsus, wing, and abdomen) were analyzed by qPCR. 【Results】The antennal transcriptomes of the *O. asiaticus* adults were successfully assembled. A total of 61 629 unigenes with a mean length of 733 nt were obtained, and the total length and N50 were 45 175 449 nt and 1 130 nt, respectively. Among them, 26 064 unigenes (42.29%) were annotated to six databases (NR, NT, Swiss-Prot, KEGG, COG and GO). A total of 17 putative CSP genes were identified by blasting, cDNA cloning and sequencing. BlastP best hit results and phylogenetic analysis both showed that the putative OasiCSPs were most closely related to LmigCSPs from *Locusta migratoria* and SgreCSPs from *Schistocerca gregaria*. qPCR analysis showed that there were significant differences in the expression levels of eight different CSP genes in different adult tissues. Especially, *OasiCSP*8 was much highly expressed in the labial palps and maxillary palps, and *OasiCSP*11 and *OasiCSP*13 had the highest expression levels in the antenna. *OasiCSP*15 had much higher expression levels in chemosensory tissues (antenna, labrum, labium without labial palps, labial palp, maxillary palp, and tarsus) than in non-chemosensory tissues (head without antenna and mouthpart, thorax, wing, and abdomen). However, *OasiCSP*12 had similar expression distribution in nearly all the tested tissues. 【Conclusion】The results suggest that OasiCSPs might play multiple roles in the chemosensory process and development of *O. asiaticus*. Our findings provide a foundation for the further functional studies of these CSPs in *O. asiaticus*.

Key words：*Oedaleus asiaticus*; antenna; transcriptome; chemosensory protein; expression profile

1 INTRODUCTION

Through chemosensory systems, insects perceive chemical signals ensuring activities such as host-seeking, feeding, mating, and ovipositing. The main olfactory proteins include odorant binding proteins (OBPs), chemosensory proteins (CSPs), olfactory receptors (ORs), odorant degrading enzymes (ODEs), and sensory neuronal membrane proteins (SNMPs) (Leal, 2013). OBPs and CSPs are two functionally similar classes of carrier proteins, which dissolve and transfer semiochemicals to chemosensory receptors. CSPs are main binding proteins in insects, and primarily bind and carry non-volatile semiochemicals whereas OBPs bind and carry volatile semiochemicals (Pelosi *et al.*, 2006). Insect CSPs have a molecular weight of about 13 kD with 100 to 115 amino acid residues and four conserved hemicarine sites, forming two disulfide bonds (Angeli *et al.*, 1999). Genetic analysis of chemosensory proteins in Diptera, Hymenoptera, Lepidoptera, Orthoptera and Coleoptera showed that CSPs have highly conserved domains, and most of the arrangement patterns of conserved cysteine sites

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are C1-X6-8-C2-X16-21-C3-X2-C4 (Briand *et al.*, 2002). The gene encoding CSPs was firstly found in the antennae of *Drosophila melanogaster*, and named olfactory specific-D (OS-D) protein gene (McKenna *et al.*, 1994). To date, a large number of CSP genes have been reported from different insect orders, including Orthoptera (Angeli *et al.*, 1999; Picimbon *et al.*, 2000; Ban *et al.*, 2003), Lepidoptera (Dani *et al.*, 2011; Qiao *et al.*, 2013; Liu NY *et al.*, 2015), Hemiptera (Jacobs *et al.*, 2005), Hymenoptera (Briand *et al.*, 2002; Zhao *et al.*, 2016), Diptera (Leitch *et al.*, 2015), Phasmatodea (Marchese *et al.*, 2000) and Homoptera (Zhou *et al.*, 2010; Zhou *et al.*, 2014). Moreover, CSPs have been found to be extensively expressed not only in chemosensory organs, including antennae, tarsus, labial palps and maxillary palps, but also in non-chemosensory organs, including legs, wings, thoraxes, abdomens, ovaries, pheromone glands and compound eyes (Maleszka and Stange, 1997; Angeli *et al.*, 1999; Picimbon *et al.*, 2001; Jin *et al.*, 2006, Gong *et al.*, 2007; Zhu *et al.*, 2015). Therefore, insect CSPs may have multiple functions in insect chemoreception, growth and development.

Identification and expression profiling of chemosensory genes are important for investigating their functions and molecular mechanisms in insect chemoreception. With the rapid development of the high-throughput sequencing techniques, RNA-Seq has been applied to identify many CSP genes in some insects whose complete genomic sequences are unavailable, such as *Cydia pomonella* (Bengtsson *et al.*, 2012), *Ips typographus* and *Dendroctonus ponderosae* (Andersson *et al.*, 2013), *Spalangia endius* (Zhang Y *et al.*, 2014), and *Helicoverpa armigera* and *H. assulta* (Zhang J *et al.*, 2015).

The band-winged grasshopper, *Oedaleus asiaticus* Bei-Bienko, is one of the most important grasshopper pests in northern China and often causes great economic losses (Kang and Chen, 1994). This species is an oligophagous herbivore, foraging mainly on gramineaceous plants (Guan and Wei, 1989). While previous studies with *O. asiaticus* focused primarily on ecology (Kang and Chen, 1995; Pang *et al.*, 2014), there is little information about its chemosensory characteristics. We previously identified 15 OBP genes from the antennal transcriptomes of *O. asiaticus*, and determined their expression profiles in the antennae, mouthparts, and wings of both males and females by RT-PCR and qPCR (Zhang S *et al.*, 2015). In the present study, we conducted a further analysis of the antennal transcriptomes of *O. asiaticus*, identified 17 CSP genes and investigated their expression profiles in different adult tissues using qPCR. We also explored the evolutionary relationship between the *O. asiaticus* CSPs and other insect CSPs. Our results provide a basis for further research on the physiological functions of CSPs in *O. asiaticus*.

2 MATERIALS AND METHODS

2.1 Insects and tissues collection

The *O. asiaticus* population was collected from the Gegentala grasslands (41°46′N, 111°49′E), Inner Mongolia in July, 2016. The different tissues, including antennae, heads without antennae and mouthparts, labra, labia without labial palps, labial palps, maxillary palps, thoraxes, wings, tarsi and abdomens, were excised from adults of both sexes, and immediately frozen in liquid nitrogen, and stored at −80℃.

2.2 cDNA library construction and Illumina sequencing

Total RNA was extracted from 30 male and 30 female adult antennae, respectively, using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. The integrity of total RNA was checked using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The mRNA was purified from 16.74 and 18.15 μg total RNA of male and female antennae, respectively, with Oligo (dT) magnetic beads. After mixed with the fragmentation buffer, the mRNA was fragmented into short fragments. The first-strand cDNA was synthesized using random hexamer-primers, followed by the second-strand cDNA synthesis using DNA polymerase, RNaseH and dNTPs. Short fragments were purified and resolved with EB buffer for end reparation and adding poly (A), and then ligated to adaptors. Subsequently, the suitable fragments were selected as the templates for PCR amplification. The cDNA library was sequenced on the HisSeq™ 2000 platform using paired-end technology in the BGI Genomics Co., Ltd.

2.3 *De novo* transcriptome assembly and function annotation

De novo transcriptome assembly was carried using the short read program Trinity (Grabherr *et al.*, 2011), and then the Trinity outputs were clustered by TGICL (Pertea *et al.*, 2003). The unigene dataset was made up of the consensus cluster sequences and singletons. The function annotation of unigenes was performed by NCBI BlastX against protein databases, including NR, Swiss-Prot, KEGG and COG, and by NCBI BlastN against nucleotide sequence (NT) database with the E-value $< 10^{-5}$. Gene names were assigned to each unigene based on the best BlastX hit with the highest score. The unigenes that could not be aligned to any of the above databases were scanned by

ESTScan program, producing nucleotide sequence (5′-3′) direction and amino sequence of the predicted coding region (Iseli *et al.*, 1999). The Blast results were then imported into Blast2GO pipeline for GO Annotation (Conesa *et al.*, 2005). Each GO ID was assigned to the related ontology entries using the WEGO software (Ye *et al.*, 2006). The longest open reading frame (ORF) for each unigene was determined using the ORF Finder tool (https://www.ncbi.nlm.nih.gov/orffinder).

2.4 Identification of chemosensory genes

"CSP" and "chemosensory proteins" were used as key words to screen the annotated sequences. Moreover, a TBlastN analysis was performed to identify putative CSP genes in *O. asiaticus* using known CSP sequences from orthopteran species as "query" to screen the transcriptome database. All putative CSP genes were manually checked using the BlastX program against the NR nucleotide database of the National Center for Biotechnology Information (NCBI) with a cut-off E-value $<10^{-5}$ (Zhou *et al.*, 2006).

To validate the reliability of the sequences, gene-specific primers were designed by Primer Premier 5.0 to amplify the ORF sequences of all putative CSP genes (Table 1). Total RNA was extracted from 40 antennae of male and female adults using TaKaRa MiniBEST Universal RNA Extraction Kit for first-strand cDNA synthesized by PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa) according to the manufacturer's instructions. The integrity and purity of RNA samples were measured using 1.5% agarose gel eletrophoresis and Nano Photometer™P-Class (IMPLEN, Germany), respectively. PCR reaction was programmed as follows: denaturation at 94℃ for 3 min, followed by 30 cycles of 94℃ for 30 s, annealing at 60℃ for 30 s (each primer uses a different annealing temperature), extension at 72℃ for 60 s, and then incubation for 10 min at 72℃. Amplified products were purified by agarose gel electrophoresis and cloned into pMD-19T vector (TaKaRa), then transformed into *Escherichia coli* DH5α competent cells (TaKaRa). The white colonies were selected and placed in LB medium containing 100 μg/mL ampicillin (Amp), oscillated overnight, and identified by PCR. The PCR products were sequenced for validation.

Table 1　Primers used in this study

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Use of primers
*CSP*1	ATGGCGATTGCGCTCTCAGCCC	TCAGGCGCTGAGCAGCTCTGGGT	cDNA cloning
*CSP*2	ATGGCCGGAAAGCTGACCGTGT	TCAGAAGTTGATGCCGCGCTGC	
*CSP*3	ATGGCGAGCAACTTGCTGGTATACT	TTAAAAGTCGACTCCGCGCTGC	
*CSP*4	ATGAAGGCCGCCCTG	TTATTGGTGGAGCTCCTT	
*CSP*5	ATGAAGGCCTCCCTGGTGCTG	TTATTGGTGGAGCTCCTTCTCGC	
*CSP*6	ATGAAGGCCGCCCTGCTCCTAGT	TCACTCGTGCAGCTCCCTCTCCC	
*CSP*7	ATGAGGAACTCCTGCTTGGCC	TCAGGAAGAGAGCTTCTTGAG	
*CSP*8	ATGAAGGCCATCCTGGTACTG	TTATTCCTTCAGTTCATCCTC	
*CSP*9	ATGAGCCGCCTCTGCTTCGCT	TCACACCTTGATGCCGTCGGC	
*CSP*10	ATGGCGGCAGCGAGGCAGCTG	CTAGAGGTTGACGCCGCG	
*CSP*11	GGATCCATGAAGGCGTCTGCTCTGCTG	CTCGAGCTAGGCCGAGAGGCG	
*CSP*12	GACGACCGCTACGCCAA	GCTCACTGTTGCTGTTG	
*CSP*13	ATGTCCCGCGTGGCGCG	TCAGGCTGTGGTTTTGA	
*CSP*14	ATGCATCGCCACAGCGCT	TTACTTCTCCTCTTCATCGTCGTCC	
*CSP*15	ATGGCCCGCTCTAGCCTCCTG	TCAGGGGATGATGCCCTT	
*CSP*16	ATGTCGGAGGGTGCCGAGG	TTACGACGAGACCTTCTTGAGTTCC	
*CSP*19	TACGACAACATCGACCTGGACGACG	TTAGGCGGAGACCCTCTTGAGCTCA	
*CSP*4	GACCGGCTGTTCGACAAGTA	GTTGTTGACGAGGTGCTTCA	qPCR
*CSP*7	GGGCAAAGAGCTCAAGAGTG	CCGTCAGGGTCGTACTTGTT	
*CSP*8	ACCACCAAGTTCGACGACAT	GGCGCACTCAGTTTCTAAGG	
*CSP*10	CTGCTCAAGAGCGTGCTG	CCTCGGGGTCGTACTTGTC	
*CSP*11	AAGTACCAGTCGCCCTACGA	CGTGCTTCTGCTTCTCAGTG	
*CSP*12	GCTACGCCAAGTACGACCAC	CACGTTCTCCTTCTGCTTGG	
*CSP*13	TACGACAGCGTGGACGTAGA	TGTTCATCGAGTGCTTCTGC	
*CSP*15	GGACGACGACTGCAATGTG	AGCTGCTTCATCTCCTCCG	
β-actin	CTACCACAGCCGAGCGAGAA	CCATCAGGCAGCTCGTAGGA	

2.5 Sequence and phylogenetic analysis

The ORFs of all putative CSP genes were determined using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder). The signal peptides were predicted using SignalP4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). The identity of amino acid sequences was calculated using an online prediction website ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Phylogenetic analysis of 17 putative CSPs from *O. asiaticus* and 91 CSPs from eight other insect species including *Aphis gossypii*, *Bombyx mori*, *Helicoverpa armigera*, *Laodelphax striatella*, *Sogatella furcifera*, *Adelphocoris lineolatus*, *Locusta migratoria*, and *Schistocerca gregaria* was performed with MEGA 6.0 software (Tamura *et al.*, 2011). The neighbor-joining method was used to create phylogenetic trees with *p*-distance under the default parameters of MEGA 6.0 program. Bootstrap analysis of 1 000 replications was performed.

2.6 Expression analysis of CSP genes

qPCR was performed to determine the expression levels of putative CSP genes in different adult tissues of *O. asiaticus*. Thirty antennae, three heads (without antennae and mouthparts), three thoraxes, three abdomens, 15 labra, 15 labia (without labial palps), 60 labial palps, 60 maxillary palps, 20 wings, and 42 tarsi were collected from adult females and males 10 – 12 days after eclosion without mating, respectively, for each biological replicate. The reference gene *β-actin* of *O. asiaticus* was used as the endogenous control to normalize the target gene expression and to correct for any sample-to-sample variation. Eight *OasiCSP*s with complete ORFs were selected for qPCR analysis. The gene-specific primers (Table 1) for qPCR were designed using Primer 5.0. qPCR analysis was conducted using FTC-3000P Real-Time PCR System (Funglyn Biotech, Canada). Each qPCR reaction was performed using BRYT Green ® dye (Go Taq ® qPCR Master Mix, Promega, America) in three biological replicates, and 10 μL reaction mixture for each sample according to the manufacturer's instructions. PCR reaction programs were as follows: initial denaturation at 95℃ for 10 min, followed by 45 cycles of 95℃ for 15 s and 60℃ for 1 min. Finally, the dissociation curve was analyzed in each reaction to confirm the specificity of the primers (reaction condition: 94℃ for 30 s, 60℃ for 90 s and 94℃ for 10 s). The relative quantification of the gene expression level among all tissues was analyzed using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001). In order to compare the expression levels of different genes in various tissues, the expression levels of genes in relative to that of *OasiCSP*10 in female tarsi, which was arbitrarily set as one, were calculated. The differences in the expression levels of CSP genes between female and male tissues were compared using Student's *t*-test. The comparative analyses of the expression level of each gene among different tissues were determined using a one-way analysis of variance (ANOVA), followed by Duncan's test in SPSS Statistics 18.0 (SPSS Inc., Chicago, IL, USA).

3 RESULTS

3.1 Transcriptome sequencing and *de novo* assembly

The RNA samples extracted from male and female antennae of the *O. asiaticus* adults were sequenced. A total of 154 125 136 and 145 802 028 raw reads were generated from the female and male antennal samples, respectively. After eliminating adaptor sequences, contaminating sequences and low quality sequences, 137 426 706 and 127 822 838 clean reads were obtained from female and male antennae, respectively. These clean reads were assembled into 125 922 contigs with a mean length of 354 nt for females and 123 313 contigs with a mean length of 318 nt for males (Table 2). After clustering and redundancy filtering, these contigs were further assembled into 71 934 unigenes and 69 134 unigenes, respectively. Finally, all clean reads from female and male antennae were assembled together into 61 629 unigenes with a mean length of 733 nt. The total length and N50 were 45 175 449 nt and 1 130 nt, respectively.

3.2 Homology analysis and functional annotation

Of all the 61 629 unigenes assembled, a total of 26 064 unigenes (42.29%) were annotated to six databases (NR, NT, Swiss-Prot, KEGG, GOG and GO), and 23 053 (37.40%), 11 867 (19.26%), 18 193 (29.52%), 15 964 (25.90%), 8 508 (13.81%) and 10 599 (17.20%) unigenes were annotated to NR, NT, Swiss-Prot, KEGG, COG and GO database, respectively.

The Gene Ontology (GO) annotation was used to classify the unigenes into the functional groups according to the GO category (Fig. 1). A total of 10 599 unigenes were assigned for GO terms based on sequence homology. For biological process, the largest number of unigenes were assigned to cellular process (6 673, 62.96%) and metabolic process (5 191, 48.98%). For cellular component, cell (4 899, 46.22%) and cell part (4 897, 46.20%) were among the most highly represented ontologies. For molecular function, the most represented ontologies were binding (5 351, 50.49%) and catalytic activity (5 219, 49.24%).

Table 2　Statistics of the assembly quality of the *Oedaleus asiaticus* antennal transcriptomes

	Sample	Total number	Total length (nt)	Mean length (nt)	N50 *	Total consensus sequences	Distinct clusters	Distinct singletons
Contig	Female antenna	125 922	44 622 274	354	665	-	-	-
	Male antenna	123 313	39 156 991	318	512	-	-	-
Unigene	Female antenna	71 934	43 458 592	604	954	71 934	11 757	60 177
	Male antenna	69 134	36 226 182	524	770	69 134	10 330	58 804
	Both	61 629	45 175 449	733	1 130	61 629	12 145	49 484

* Given a set of contigs, N50 is defined as the sequence length of the shortest contig at 50% of the total genome length.

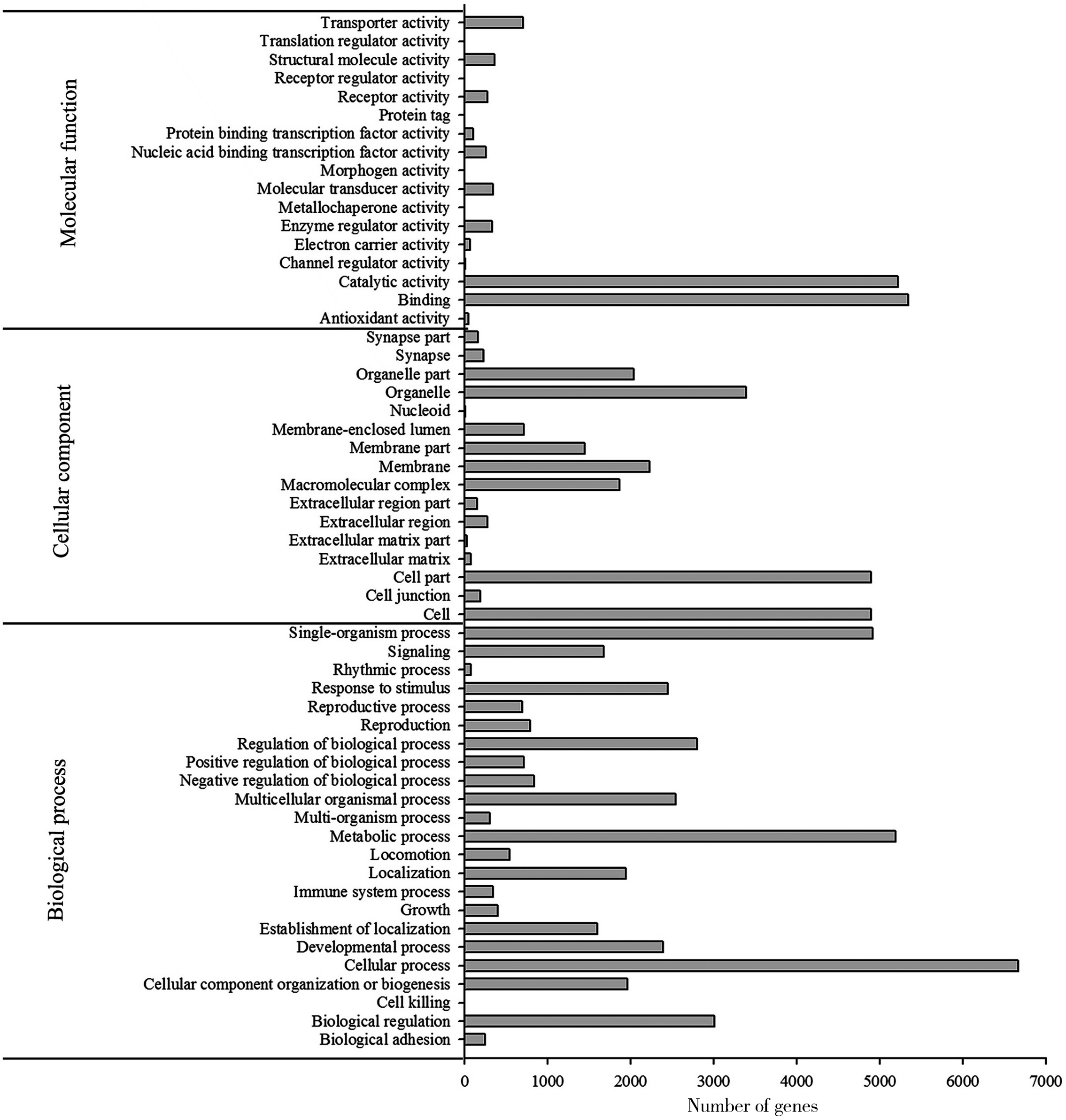
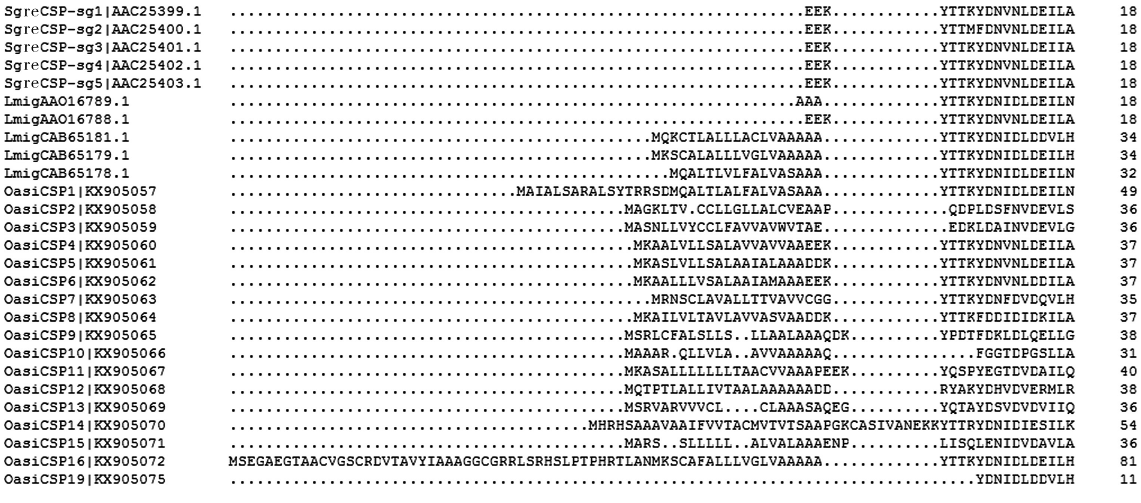


Fig. 1　Gene ontology (GO) classification of the *Oedaleus asiaticus* antennal transcripts with Blast2GO program

3.3　Identification and sequence analysis of CSPs

From the antennal transcriptomes of *O. asiaticus*, 20 putative CSP-encoding unigenes, named as *OasiCSP*1 - *OasiCSP*20, were identified. These 20 unigenes, which were identified as CSP section fragments by Blasting, were cloned. The sequence alignment of the above unigenes using DNAMAN v6.0 (Lynnon Biosoft, Canada) showed that the overall nucleotide sequence identities of *OsiaCSP*1 - 16 and *OsiaCSP*19 were quite high, ranging from 96.38% to 100%, and only a few nucleotide replacements occur between the cloned sequences and candidate sequences of *OsiaCSP*1 – 3, *OsiaCSP*5 – 10, *OsiaCSP*16 and *OsiaCSP*19. However, the nucleotide sequence identity of *OasiCSP*17/18/20 was less than 40% between the cloned sequences and CSP-encoding unigenes screened from the antennal transcriptomes, which may be only partial sequences of some *OasiCSP* genes. Therefore, 17 CSP genes were finally confirmed.

By BlastP matching of the CSPs, the best BlastP matching species was *L. migratoria* for 16 OasiCSPs except for OasiCSP8 whose best BlastP matching species was *S. gregaria* (Table 3). Bioinformatics analysis indicated that, except for *OasiCSP*19, the other 16 *OasiCSP* genes have complete ORFs ranging from 372 bp to 516 bp and encode 124 – 172 amino acids with a calculated molecular weight of 11.60 – 18.72 kD. Multiple alignment of the amino acid sequences of all the 17 OasiCSPs indicated that these OasiCSPs contain four conserved cysteine residues, meeting the following characteristics: C1-X6-8-C2-X18-C3-X2-C4, and share the conserved characteristics of typical insect CSP family (Fig. 2).


SgreCSP-sg1|AAC25399.1 ....EEK...........YTTKYDNVNLDEILA 18
SgreCSP-sg2|AAC25400.1 ....EEK...........YTTMFDNVNLDEILA 18
SgreCSP-sg3|AAC25401.1 ....EEK...........YTTKYDNVNLDEIIA 18
SgreCSP-sg4|AAC25402.1 ....EEK...........YTTKYDNVNLDEILA 18
SgreCSP-sg5|AAC25403.1 ....EEK...........YTTKYDNVNLDEILA 18
LmigAAO16789.1 ....AAA............YTTKYDNIDLDEILN 18
LmigAAO16788.1 ....EEK...........YTTKYDNVNLDEILA 18
LmigCAB65181.1 ....MQKCTLALLLACLVAAAAA............YTTKYDNIDLDDVLH 34
LmigCAB65179.1 ....MKSCALALLLVGLVAAAAA............YTTKYDNIDLDEILH 34
LmigCAB65178.1 ....MQALTLVLFALVASAAA............YTTKYDNIDLDEILN 32
OasiCSP1|KX905057 ....MAIALSARALSYTRRSDMQALTLALFALVASAAA............YTTKYDNIDLDEILN 49
OasiCSP2|KX905058 ....MAGKLTV.CCLLGLLALCVEAAP............QDPLDSFNVDEVLS 36
OasiCSP3|KX905059 ....MASNLLVYCCLFAVVAVWVTAE............EDKLDAINVDEVLG 36
OasiCSP4|KX905060 ....MKAALVLLSALAVVAVVAAEEK...........YTTKYDNVNLDEILA 37
OasiCSP5|KX905061 ....MKASLVLLSALAAIALAAADDK...........YTTKYDNVNLDEILA 37
OasiCSP6|KX905062 ....MKAALLLVSALAAIAMAAAEEK...........YTTKYDNVNLDDILA 37
OasiCSP7|KX905063 ....MRNSCLAVALLTTVAVVCGG...........YTTKYDNFDVDQVLH 35
OasiCSP8|KX905064 ....MKAILVLTAVLAVVASVAADDK...........YTTKFDDIDIDKILA 37
OasiCSP9|KX905065 ....MSRLCFALSLLS..LLAALAAAQDK.........YPDTFDKLDLQELLG 38
OasiCSP10|KX905066 ....MAAAR.QLLVLA..AVVAAAAAQ...............FGGTDPGSLLA 31
OasiCSP11|KX905067 ....MKASALLLLLLLTAACVVAAAPEEK.........YQSPYEGTDVDAILQ 40
OasiCSP12|KX905068 ....MQTPTLALLIVTAALAAAAAADD...........RYAKYDHVDVERMLR 38
OasiCSP13|KX905069 ....MSRVARVVVCL....CLAAASAQEG.........YQTAYDSVDVDVIIQ 36
OasiCSP14|KX905070 ....MHRHSAAAVAAIFVVTACMVTVTSAAPGKCASIVANEKKYTTRYDNIDIESILK 54
OasiCSP15|KX905071 ....MARS..SLLLLL..ALVALAAAENP..........LISQLENIDVDAVLA 36
OasiCSP16|KX905072 MSEGAEGTAACVGSCRDVTAVYIAAAGGCGRRLSRHSLPTPHRTLANMKSCAFALLLVGLVAAAAA............YTTKYDNIDLDEILH 81
OasiCSP19|KX905075 ....YDNIDLDDVLH 11

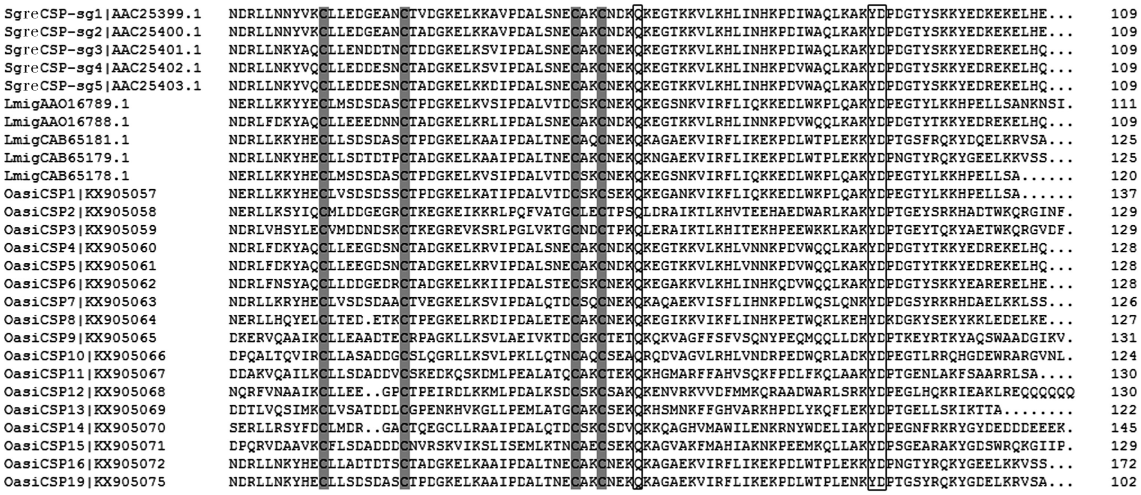

SgreCSP-sg1|AAC25399.1 NDRLLNNYVKCLLEDGEANCTVDGKELKKAVPDALSNECAKCNDKQKEGTKKVLKHLINHKPDIWAQLKAKYDPDGTYSKKYEDKEKELHE... 109
SgreCSP-sg2|AAC25400.1 NDRLLNNYVKCLLEDGEANCTADGKELKKAVPDALSNECAKCNDKQKEGTKKVLKHLINHKPDIWAQLKAKYDPDGTYSKKYEDKEKELHE... 109
SgreCSP-sg3|AAC25401.1 NDRLLNKYAQCLLENDDTNCTDDGKELKSVIPDALSNECAKCNEKQKEGTKKVLKHLINHKPDIWAQLKAKYDPDGTYSKKYEDREKELHQ... 109
SgreCSP-sg4|AAC25402.1 NDRLLNKYVQCLLEDDESNCTADGKELKSVIPDALSNECAKCNEKQKEGTKKVLKHLINHKPDVWAQLKAKYDPDGTYSKKYEDREKELHQ... 109
SgreCSP-sg5|AAC25403.1 NDRLLNKYVQCLLEDDESNCTADGKELKKDIPDALSNECAKCNEKQKEGTKKVLKHLINHKPDVWAQLKAKYDPDGTYSKKYEDREKELHQ... 109
LmigAAO16789.1 NERLLKKYYECLMSDSDASCTPDGKELKVSIPDALVTDCSKCNEKQKEGSNKVIRFLIQKKEDLWKPLQAKYDPEGTYLKKHPELLSANKNSI. 111
LmigAAO16788.1 NDRLFDKYAQCLLEEEDNNCTADGKELKRLIPDALSNECAKCNDKQKEGTKKVLRHLINNKPDVWQQLKAKYDPDGTYTKKYEDREKELHQ... 109
LmigCAB65181.1 NDRLLKKYHECLLSDSDASCTPDGKELKAAIPDALTNECAQCNEKQKAGAEKVIRFLIKEKPDLWTPLEKKYDPTGSFRQKYDQELKRVSA... 125
LmigCAB65179.1 NDRLLNKYHECLLSDTDTPCTADGKELKAAIPDALTNECAKCNEKQKNGAEKVIRFLIKEKPDLWTPLEKKYDPNGTYRQKYGEELKKVSS... 125
LmigCAB65178.1 NERLLKKYHECLMSDSDASCTPDGKELKVSIPDALVTDCSKCNEKQKEGSNKVIRFLIQKKEDLWKPLQAKYDPEGTYLKKHPELLSA...... 120
OasiCSP1|KX905057 NERLLKKYHECLVSDSDSSCTPDGKELKATIPDALVTDCSKCSEKQKEGANKVIKFLIQKKEDLWKPLQAKYDPEGTYLKKHPELLSA...... 137
OasiCSP2|KX905058 NERLLKSYIQCMLDDGEGRCTKEGKEIKKRLPQFVATGCLECTPSQLDRAIKTLKHVTEEHAEDWARLKAKYDPTGEYSRKHADTWKQRGINF. 129
OasiCSP3|KX905059 NDRLVHSYLECVMDDNDSKCTKEGREVKSRLPGLVKTGCNDCTPKQLERAIKTLKHITEKHPEEWKKLKAKYDPTGEYTQKYAETWKQRGVDF. 129
OasiCSP4|KX905060 NDRLFDKYAQCLLEEGDSNCTADGKELKRVIPDALSNECAKCNDKQKEGTKKVLKHLVNNKPDVWQQLKAKYDPDGTYTKKYEDREKELHQ... 128
OasiCSP5|KX905061 NDRLFDKYAQCLLEEGDSNCTADGKELKRVIPDALSNECAKCNDKQKEGTKKVLKHLVNNKPDVWQQLKAKYDPDGTYTKKYEDREKELHQ... 128
OasiCSP6|KX905062 NDRLFNSYAQCLLDDGEDRCTADGKELKKIIPDALSTECSKCNEKQKEGAKKVLKHLINHKQDVWQQLKAKYDPDGTYSKKYEARERELHE... 128
OasiCSP7|KX905063 NDRLLKRYHECLVSDSDAACTVEGKELKSVIPDALQTDCSQCNEKQKAQAEKVISFLIHNKPDLWQSLQNKYDPDGSYRKRHDAELKKLSS... 126
OasiCSP8|KX905064 NERLLHQYELCLTED.ETKCTPEGKELRKDIPDALETECAKCNEKQKEGIKKVIKFLINHKPETWQKLKEHYDKDGKYSEKYKKLEDELKE... 127
OasiCSP9|KX905065 DKERVQAAIKCLLEAADTECRPAGKLLKSVLAEIVKTDCGKCTETQKQKVAGFFSFVSQNYPEQMQQLLDKYDPTKEYRTKYAQSWAADGIKV. 131
OasiCSP10|KX905066 DPQALTQVIRCLLASADDGCSLQGRLLKSVLPKLLQTNCAQCSEAQRQDVAGVLRHLVNDRPEDWQRLADKYDPEGTLRRQHGDEWRARGVNL. 124
OasiCSP11|KX905067 DDAKVQAILKCLLSDADDVCSKEDKQSKDMLPEALATQCAKCTEKQKHGMARFFAHVSQKFPDLFKQLAAKYDPTGENLAKFSAARRLSA.... 130
OasiCSP12|KX905068 NQRFVNAAIKCLLEE..GPCTPEIRDLKKMLPDALKSDCSKCSAKQKENVRKVVDFMMKQRAADWARLSRKYDPEGLHQKRIEAKLREQQQQQQ 130
OasiCSP13|KX905069 DDTLVQSIMKCLVSATDDLCGPENKHVKGLLPEMLATGCAKCSEKQKHSMNKFFGHVARKHPDLYKQFLEKYDPTGELLSKIKTTA........ 122
OasiCSP14|KX905070 SERLLRSYFDCLMDR..GACTQEGCLLRAAIPDALQTDCSKCSDVQKKQAGHVMAWILENKRNYWDELIAKYDPEGNFRKRYGYDEDDDEEEK. 145
OasiCSP15|KX905071 DPQRVDAAVKCFLSDADDDCNVRSKVIKSLISEMLKTNCAECSEKQKAGVAKFMAHIAKNKPEEMKQLLAKYDPSGEARAKYGDSWRQKGIIP. 129
OasiCSP16|KX905072 NDRLLNKYHECLLADTDTSCTADGKELKAAIPDALTNECAKCNEKQKAGAEKVIRFLIKEKPDLWTPLEKKYDPNGTYRQKYGEELKKVSS... 172
OasiCSP19|KX905075 NDRLLNKYHECLLSDSDASCTPDGKELKAAIPDALTNECAKCNEKQKAGAEKVIRFLIKEKPDLWTPLENKYDPTGSYRQKYGDELKRVSA... 102

Fig. 2 Multiple alignment of amino acid sequences of OasiCSPs with other insect CSPs

The four conserved cysteines are labeled in grey, and other conserved residues are labeled in box. The insect species are as follows: *Schistocerca gregaria* (Sgre), *Locusta migratoria* (Lmig), and *Oedaleus asiaticus* (Oasi).

3.4 Phylogenetic analysis

A phylogenetic tree (neighbor-joining tree) was constructed to display the genetic relationships among these CSP related proteins, including 17 CSPs of *O. asiaticus* and 91 CSPs of other 8 insect species, based on their amino acid sequences. Eight OasiCSPs (OasiCSP1, OasiCSP4 – 8, OasiCSP16 and OasiCSP19) were clustered together into one large clade with all CSPs from two orthopteran species, *L. migratoria* and *S. gregaria*, indicating that these eight OasiCSPs have the highest amino acid sequence identity with LmigCSPs and SgreCSPs from the same order species, while other OasiCSPs were clustered into distinct clades with all CSPs from other six species

Table 3　Physico-chemical predictions and the BlastX match of the putative chemosensory proteins from *Oedaleus asiaticus* (OasiCSPs)

Gene	GenBank accession no.	Amino acid length (aa)	Complete ORF	Molecular weight (kD)	Isoelectric point	Size of signal peptide (aa)	Best BlastX match				
							Description	GenBank accession no.	Species	E value	Amino acid sequence identity (%)
*CSP*1	KX905057	137	Yes	15.28	7.29	None	Chemosensory protein	CAB65178.1	*Locusta migratoria*	3.00E−78	93
*CSP*2	KX905058	129	Yes	14.54	6.74	20	Hypothetical protein	CAJ01467.1	*Locusta migratoria*	1.00E−81	89
*CSP*3	KX905059	129	Yes	14.78	6.26	21	Hypothetical protein, partial	CAJ01475.1	*Locusta migratoria*	4.00E−60	91
*CSP*4	KX905060	128	Yes	14.50	5.82	19	CSP precursor, partial	AAO16785.1	*Locusta migratoria*	8.00E−72	96
*CSP*5	KX905061	128	Yes	14.46	5.81	19	CSP precursor, partial	AAO16785.1	*Locusta migratoria*	1.00E−70	94
*CSP*6	KX905062	128	Yes	14.50	6.26	19	CSP precursor, partial	AAO16783.1	*Locusta migratoria*	3.00E−63	85
*CSP*7	KX905063	126	Yes	14.22	6.90	19	Hypothetical protein, partial	CAJ01466.1	*Locusta migratoria*	4.00E−52	73
*CSP*8	KX905064	127	Yes	14.74	5.37	19	Chemosensory protein CSP-sg5, partial	AAC25403.1	*Schistocerca gregaria*	5.00E−41	63
*CSP*9	KX905065	131	Yes	14.63	6.49	20	Hypothetical protein, partial	CAJ01469.1	*Locusta migratoria*	4.00E−66	86
*CSP*10	KX905066	124	Yes	13.38	7.29	19	Hypothetical protein, partial	CAJ01462.1	*Locusta migratoria*	1.00E−25	38
*CSP*11	KX905067	130	Yes	14.16	6.50	20	Hypothetical protein, partial	CAJ01474.1	*Locusta migratoria*	6.00E−27	48
*CSP*12	KX905068	131	Yes	15.03	9.56	21	Hypothetical protein, partial	CAJ01470.1	*Locusta migratoria*	1.00E−74	90
*CSP*13	KX905069	122	Yes	13.43	7.37	18	Hypothetical protein, partial	CAJ01474.1	*Locusta migratoria*	9.00E−26	43
*CSP*14	KX905070	145	Yes	16.41	5.30	25	Hypothetical protein	CAJ01476.1	*Locusta migratoria*	1.00E−77	92
*CSP*15	KX905071	129	Yes	14.06	7.14	18	Hypothetical protein, partial	CAJ01462.1	*Locusta migratoria*	3.00E−72	94
*CSP*16	KX905072	172	Yes	18.72	7.70	None	Chemosensory protein	CAB65179.1	*Locusta migratoria*	1.00E−82	97
*CSP*19	KX905075	102	No	11.60	4.94	None	CSP precursor	AAO16797.1	*Locusta migratoria*	9.00E−66	95

(Fig. 3). Interestingly, five OasiCSPs (OasiCSP9 – 11, OasiCSP13, and OasiCSP15) were clustered into one clade alone, OasiCSP2 and OasiCSP3 were grouped into one sole subclade, and OasiCSP12 and OasiCSP14 were separated into two distinct branches.

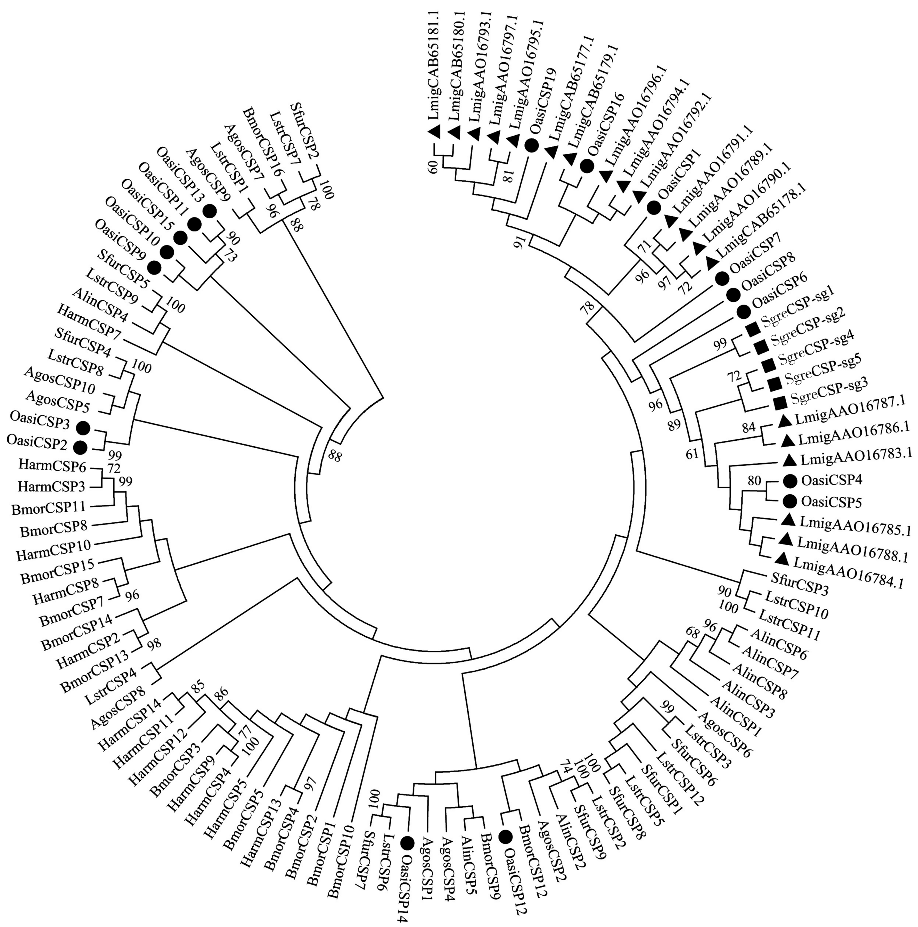


Fig. 3 Phylogenetic relationships based on the amino acid sequence of CSPs from *Oedaleus asiaticus* and other insect species using neighbor-joining method

Phylogenetic tree based on amino acid sequences of CSPs from *Oedaleus asiaticus* (Oasi), *Aphis gossypii* (Agoss), *Bombyx mori* (Bmor), *Helicoverpa armigera* (Harm), *Laodelphax striatella* (Lstri), *Sogatella furcifera* (Sfur), *Adelphocoris lineolatus* (Alin), *Locusta migratoria* (Lmig), and *Schistocerca gregaria* (Sgre). The *O. asiaticus*, *L. migratoria*, and *S. gregaria* CSPs are represented by black circle, triangle, and quadrangle, respectively. The bootstrap value was based on 1 000 replicates.

3.5 Tissue-specific expression profiling of CSP genes detected by qPCR

Eight representative CSP genes, including *OasiCSP*4, *OasiCSP*7 – 8, *OasiCSP*10 – 13 and *OasiCSP*15, were selected for expression profiling in antennae, heads without antennae and mouthparts, labra, labia without labial palps, labial palps, maxillary palps, thoraxes, tarsi, wings and abdomens of both male and female adults. The expression levels of these CSP genes in different adult tissues showed significant differences (Fig. 4). Using the expression level of *OasiCSP*10 in female tarsi as the control, the relative expression levels of *OasiCSP*4, *OasiCSP*8, and *OasiCSP*15 were much higher than those of *OasiCSP*10 and *OasiCSP*12 in most tissues. The relative expression levels of *OasiCSP*11 and *OasiCSP*13 in antennae were over 900-fold higher than those in the other tissues. The relative expression levels of *OasiCSP*15 in chemosensory tissues (antenna, labrum, labium without labial palps, labial palp, maxillary palp, and tarsus) were over 2 000-fold higher than those in non-chemosensory tissues (head without antenna and mouthpart, thorax, wing, and abdomen). The relative expression levels of *OasiCSP*8 in the labial palps and maxillary palps were over 6 000-fold higher than those in other tissues. However, *OasiCSP*12 had similar expression distribution in nearly all the tested tissues. The relative expression levels of all the eight CSP genes in most tissues had significant difference between adult males and females, and especially, those of *OasiCSP*11 and *OasiCSP*13 were higher in male antennae than in female antennae.

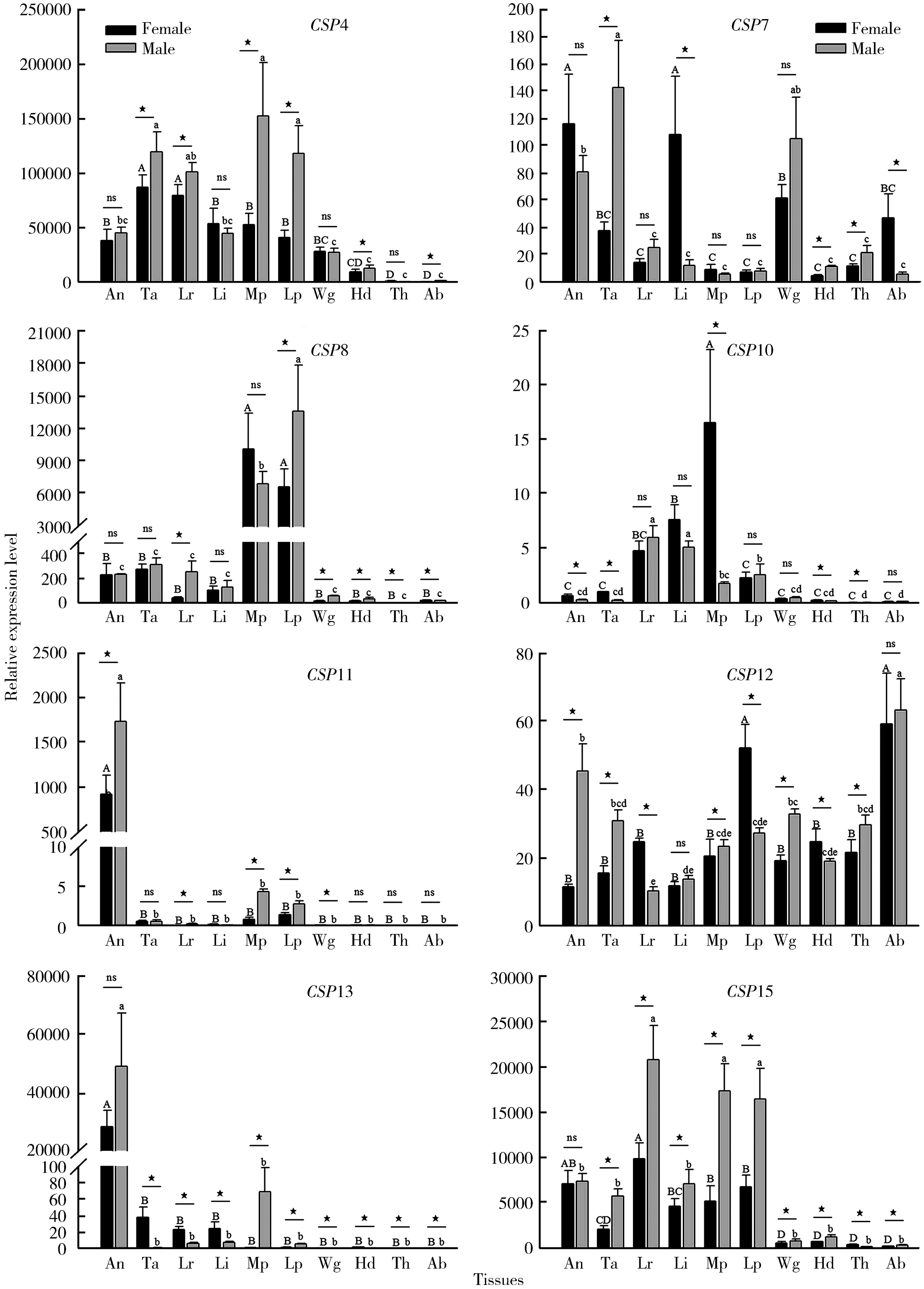


Fig. 4　Relative expression levels of CSP genes in different tissues of *Oedaleus asiaticus* male and female adults

An: Antennae; Ta: Tarsus; Lr: Labrum; Li: Labium without labial palp; Mp: Maxillary palp; Lp: Labial palp; Wg: Wing; Hd: Head without antenna and mouthpart; Th: Thorax; Ab: Abdomen. *β-actin* was used as the reference gene to normalize the target gene expression, and the expression levels of genes in relative to that of *OasiCSP*10 in female tarsi was calculated. Error bars represent the standard error of three independent experiments. Different capital and small letters above bars indicate significant difference among different tissues of females and males, respectively (Duncan's test; $P < 0.05$). The asterisk above bars indicates significant difference in the same tissue between males and females ($^{*}P < 0.05$; $^{\mathrm{ns}}$No significant difference; *t*-test).

4 DISCUSSION

To better understand the olfactory genes, we constructed antennal transcriptomes of *O. asiaticus*, and 17 CSP genes were identified. The number of CSP genes identified from the insect genomes and transcriptomes was highly variable, such as four CSP genes in *Calliphora stygia* (Leitch *et al*., 2015), six in *Dendroctonus valens* (Gu *et al*., 2015), 12 in *Tenebrio molitor* (Zhu *et al*., 2013) and *Colaphellus bowringi* (Li *et al*., 2015), 18 and 17 in *H. armigera* and *H. assulta*, respectively (Zhang J *et al*., 2015), 22 in *Cnaphalocrocis medinalis* (Liu S *et al*., 2015), 43 in *Aedes aegypti* (Pelletier and Leal, 2011), and 70 in *Locusta migratoria* (Zhou *et al*., 2013).

Phylogenetic analysis showed that eight OasiCSPs were closely related to all LmigCSPs from *L. migratoria* and SgreCSPs from *S. gregaria* in genetic relationship while other OasiCSPs were not closely related to LmigCSPs and SgreCSPs, suggesting that the CSP genes of these three grasshopper species share more recent common ancestor gene, but have diversified in order to adapt to different environments (Zhang S *et al*., 2015).

The expression characteristics of insect chemosensory proteins are extensive and diverse. The tissue-specific expression profiling of CSPs provides a way to predict their functions. In the present study, the tissue expression distribution of eight *OasiCSP*s was investigated by qPCR. In insects, different CSP genes in different tissues have different expression patterns, which are closely related to their functions, such as embryo development in *Apis mellifera* (Maleszka *et al*., 2007), female survival and reproduction in *Spodoptera exigua* (Gong *et al*., 2012), and high binding ability to the female sex pheromones in *Sesamia inferens* (Zhang YN *et al*., 2014). CSPs are enriched in chemosensory organs, such as antenna, labial palp, maxillary palp and tarsus, suggesting that these proteins may be involved in the sensing of chemical information (Maleszka and Stange, 1997; Jin *et al*., 2005; Gu *et al*., 2012; Liu *et al*., 2012). In this study, the relative expression levels of *OasiCSP*15 were much higher in chemosensory tissues (antenna, labrum, labium, labial palp, maxillary palp and tarsus) than in non-chemosensory tissues (head without antenna and mouthpart, thorax, and abdomen), implying its possible importance in olfaction and gustation. We found that the expression levels of *OasiCSP*11 and *OasiCSP*13 were exceptionally high in the antennae of both genders and much weaker in the other tested tissues, suggesting that these two CSPs may be involved in the olfactory processes (Li *et al*., 2015), and moreover, also higher in the antennae of males than in the antennae of females, suggesting that these genes are more likely related to sex-specific behaviors with a potential role as a sex pheromone sensing protein (Li *et al*., 2018). Antenna-specific CSPs were also reported in other insect species, such as *L. migratoria* (Ban *et al*., 2003), *Adelphocoris lineolatus* (Sun *et al*., 2015) and *Microplitis mediator* (Peng *et al*., 2017). The expression levels of *OasiCSP*8 were much higher in the labial palps and maxillary palps than in the other tested tissues, suggesting that this gene might play key roles in gustation because both sensilla basiconica and sensilla chaetica were found in labial palps (Jin *et al*., 2006). *OasiCSP*12 had similar expression profiles in both sensory tissues and non-sensory tissues with higher expression levels in male antennae, female labial palps, and female and male abdomens, suggesting that this gene might be involved not only in chemoreception but also in other physiological functions, such as solubilising hydrophobic pheromones produced by the pheromone glands and releasing them into the environment (Jacquin-Joly *et al*., 2001).

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亚洲小车蝗触角转录组及化学感受蛋白基因表达谱分析

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摘要:【目的】化学感受蛋白(chemosensory proteins, CSPs)是一类小分子的可溶性蛋白,在昆虫中发挥多种作用。本研究旨在组装中国北方主要草原害虫之一——亚洲小车蝗 *Oedaleus asiaticus* 的触角转录组,鉴定出化学感受蛋白基因以及分析其在成虫不同组织中的表达水平。【方法】利用 RNA-Seq 对亚洲小车蝗成虫触角进行转录组测序和组装;通过筛选转录组数据库、克隆及测序,鉴定出化学感受蛋白基因;应用 qPCR 分析 CSP 基因在成虫不同组织(触角、去除触角和口器的头、上唇、去掉下唇须的下唇、下唇须、下颚须、胸部、跗节、翅和腹部)中的表达模式。【结果】成功构建了亚洲小车蝗成虫触角转录组,共获得 61 629 个 unigenes,平均长度为 733 nt,总长度和 N50 分别为 45 175 449 和 1 130 nt。其中 26 064 个 unigenes(42.29%)注释到 6 个数据库(NR, NT, Swiss-Prot, KEGG, COG 和 GO)。通过 Blast 验证、克隆和测序鉴定出 17 个 CSP 基因;BlastP 和系统发育分析表明亚洲小车蝗 CSPs(OasiCSPs)与东亚飞蝗 *Locusta migratoria* CSPs(LmigCSPs)和沙漠蝗 *Schistocerca gregaria* CSPs(SgreCSPs)关系最密切。qPCR 分析表明,8 个 CSP 基因在成虫不同组织中的表达水平存在显着差异,特别是,*OasiCSP*8 在下唇须和下颚须中高表达,而 *OasiCSP*11 和 *OasiCSP*13 在触角中表达量最高。*OasiCSP*15 在化学感受器官(触角、上唇、去掉下唇须的下唇、下唇须、下颚须和跗节)中的表达量远高于非化学感受器官(去掉触角和口器的头部、胸部、翅和腹部)中的表达量,而 *OasiCSP*12 在几乎所有测定的组织中具有相似的表达分布。【结论】OasiCSPs 在亚洲小车蝗化学感受和发育过程中可能起着多种作用,这些结果为进一步研究这些化学感受蛋白在亚洲小车蝗中的功能奠定了基础。

关键词: 亚洲小车蝗; 触角; 转录组; 化学感受蛋白; 表达谱

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